Kinetic Partial Resolution of Racemic 4-Hydroperoxy-Cyclophosphamide by Lipase in Vinyl Acetate (Commemoration Issue Dedicated to Professor Shigeo Tanimoto On the Occation of His Retirement)

AUTHOR(S):

Baba, Naomichi; Ikeda, Kazuyuki; Iwasa, Junkichi

CITATION:

Baba, Naomichi ...[et al]. Kinetic Partial Resolution of Racemic 4-Hydroperoxy-Cyclophosphamide by Lipase in Vinyl Acetate (Commemoration Issue Dedicated to Professor Shigeo Tanimoto On the Occation of His Retirement). Bulletin of the Institute for Chemical Research, Kyoto University 1992, 70(3): 326-328

ISSUE DATE:

1992-10-30

URL:

http://hdl.handle.net/2433/77458

Bull. Inst. Chem. Res., Kyoto Univ., Vol. 70, No. 3, 1992

COMMUNICATION

Kinetic Partial Resolution of Racemic 4-Hydroperoxy-Cyclophosphamide by Lipase in Vinyl Acetate

Naomichi BABA*, Kazuyuki IKEDA** and Junkichi IWASA*

Received June 29, 1992

KEY WORDS: Lipase / Cyclophosphamide / Kinetic Resolution / Hydroperoxide

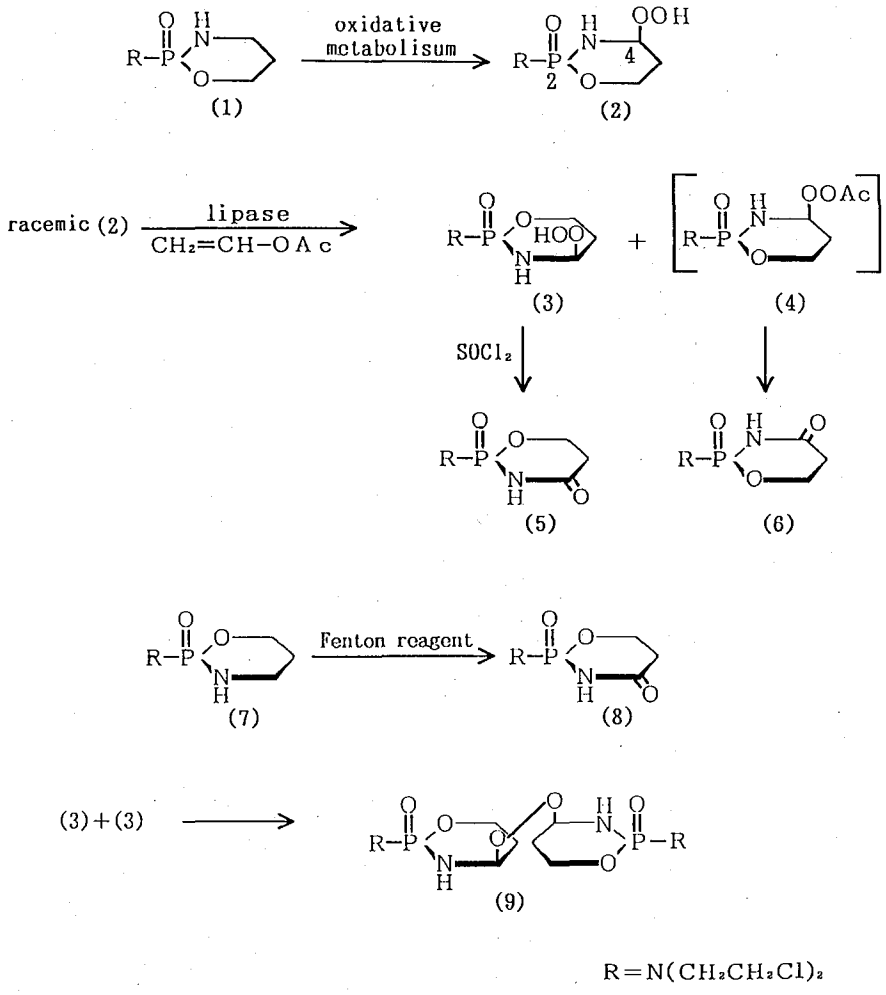
In nonaqueous media, lipases can catalyze acylation of various nucleophiles. In this category, we have reported kinetic resolution of racemic hydroperoxide such as 1-phenylethylhydroperoxide[1] and methyl 13-hydroperoxy-(9*Z*, 11*E*)-octadecadienoate[2] by use of lipases.

This communication describes application of such lipase-catalyzed acylation to kinetic resolution of 4-hydroperoxycyclophosphamide (**2**) which is a highly antitumor-active metabolic product from cyclophosphamide (**1**) in mammals[3]. Takamizawa reported a preparation of racemic form of this compound from homoallyl alcohol, bis(2-chloroethyl)amine hydrochloride and phosphorus oxychloride via ozonization and oxidative cleavage with hydrogen peroxide[3]. Optically active form of this compound, however, has not been prepared so far.

In the present study, stereoselective acetylation of this 4-hydroperoxycyclophosphamide was conducted in vinyl acetate since the ester was found to be a best medium as an acyclation reagent as well as a solvent. Typically, to a solution of (**2**) (490 mg, 1.7 mmol) in vinyl acetate (50 ml) was added lipase Amano P from *Pseudomonas fluorescens* (1.25 g) and stirred at around 40 ℃ for 1 hr. TLC analysis {silica gel, acetone/chloroform (1:2)} of the reaction mixture showed that three kinds of product A, B and C were formed with Rf value of 0.39, 0.66 and 0.85 respectively. The product (A) was found to be 4-oxocyclophosphamide (**6**) by NMR analysis and comparing it with that reported by Takamizawa[3]. This oxo-form production indicated that the hydroperoxy group of (**2**) was once acetylated to afford very unstable intermediate (**4**). It is generally known that when primary or secondary hydroperoxy group was acylated, the carbon atom bearing the group is

* 馬場直道, 岩佐順吉: Faculty of Agriculture, Okayama University, Tsushimanaka 1-1-1, Okayama 700

** 池田和幸: Present Address: Fumakilla Co. Ltd., Ohno-cho, Saekigun, Hiroshima 739-04



promptly converted to aldehyde or ketone respectively[4]. Accordingly, if the acylation step from (**2**) to (**4**) by the lipase is stereoselective to some extent, the oxo-form (**6**) having chiral phosphorus atom is expected to be partially optically active. The oxo-form (**6**), however, could not be purified completely free from (**3**) and its reliable optical rotation was unknown. On the other hand, the hydroperoxide (**3**) left unreacted was isolated pure (61 mg, 12 %) which had optical activity of $[\alpha]_D^{26}$-12.7° (c 1.04, $CHCl_3$). Since the optical purity and absolute configurations of the phosphorus and carbon atoms bearing hydroperoxy group are unknown, following stereochemical correlation experiment was conducted. First, optically active (*R*)-cyclophosphamide (**7**) with 100% optical purity was prepared according to Kinas et al[5] and it was converted to (*R*)-4-oxo-form (**8**)[6] which had $[\alpha]_D^{25}$-35.5° (c 2.07, $CHCl_3$). The recovered hydroperoxide (**3**) was also converted to the optically active oxo-form (**5**) by thionyl chloride[3] which showed $[\alpha]_D^{25}$-12.1° (c 1.21, $CHCl_3$) (34 % optical purity). Comparing these results, the oxo-form (**5**) was found to

have (*R*)-configuration at phosphorus atom. Therefore, the absolute configuration of the carbon atom bearing hydroperoxy group of (**3**) was assigned to (*R*) since it was known from X-ray analysis that oxo-group at phosphorus and hydroperoxy hydrogen were on the same side of the ring[7]. Thus, it was concluded that the hydroperoxide (**3**) left unreacted was shown to have (2*R*, 4*R*)-configuration with 34 % e.e. This outcome indicated that racemic 4-hydroperoxycyclophosphamide (**2**) was also served as a substrate to lipase in spite of the fact that structure of the hydroperoxide was very different from those reported by us[1,2]. It was also indicated that the acetylation was partially enantioselective predominating (2*S*, 4*S*)-form of (**2**).

The product (C) was shown to be a peroxide dimer (**9**) by NMR analysis[6] which was also optical active with $[\alpha]_D^{25}$-28.5° (c 1.46, $CHCl_3$). This dimer was found to be formed sponteneously under the same conditions without the lipase. Therefore, its optical form (**9**) was considered to be produced from optically active (**3**). The NMR spectrum of the product (B) was very similar to that of (C) but the structure was unknown.

In sumary, lipase Amano P was found to catalyze stereoselective acetylation of racemic 4-hydroperoxycyclophosphamide leaving optically active (2*R*, 4*R*)-species. Although the stereoselectvity is not satisfactory, the present example suggested that a possible route to optically pure form of the hydroperoxide which is an important medicinal reagent for anticancer researches.

REFERENCES

1) N. Baba, M. Mimura, J. Hiratake, K. Uchida and J. Oda, *Agric. Biol. Chem.*, **52**, 2685 (1988).
2) N. Baba, K. Tateno, J. Iwasa and J. Oda, *Agric. Biol. Chem.*, **54**, 3349 (1990).
3) A. Takamizawa, S. Matsumoto, T. Iwata, Y. Tochino, K. Katagiri, K. Yamaguchi and O. Shiratori, *J. Med. Chem.*, **18**, 376 (1975).
4) E. Hedaya and S. Winstein, *Tetrahedron Lett.*, **1962**, 563.
5) R. Kinas, K. Pankiewicz and W. J. Stec, *Bull. Acad. Pol. Sci., Ser. Sci. Chim.*, **23**, 981 (1975).
6) R. F. Struck, M. C. Thorp, W. C. Coburn, Jr. and W. R. Laster Jr., *J. Am. Chem. Soc.*, **96**, 313 (1974).
7) A. Camerman and H. W. Smith, *Biochem. Biophys. Res. Commun.*, **65**, 828 (1975).